Current Botany 2017, 8: 159-162
http://scienceflora.org/journals/index.php/cb/
doi: 10.19071/cb.2017.v8.3317

Original Article

A study of concentration of sugar mill effluents on properties of soil and types of microorganisms present in the soil

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Received: 17.10.2017
Accepted: 27.11.2017
Published: 13.12.2017

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ABSTRACT

The work was undertaken to assess the effect of various concentrations such as 0%, 25%, 50%, and 100% of sugar mill effluents (SME) on soil properties and types of microorganisms present were also studied. It has been observed that increase in concentration of sugar mill effluents causes increase in various properties of soil like total organic carbon, pH, electrical conductivity (EC),Ca and Mg ions etc.,

KEY WORDS: Electrical conductivity, microorganisms, sugar mill effluents, total organic carbon

INTRODUCTION

In our country, India, sugar industry is one of the most important agro-based industries and plays an important role in the rural economy of the country by the creation of employment. The effluents are causing odor nuisance during decomposition. Simultaneously, the by-products of sugar mills are also being used as raw materials in different industries (Usman and Gameh, 2008) and this is another advantage of sugar industry besides economic growth of the country (Kumar, 2014a; 2014b). Sugar industry is seasonal in nature, and it is generally operative for 120–200 days around the year (from November to April). There are around 650 or more sugar industries in India (ISMA, 2014; Mane *et al.*, 2015). Significant quantity of waste is generated during the manufacture of sugar and sugar mill effluents (SME) with its high values of biological oxygen demand and chemical oxygen demand which reduce available oxygen supply causing danger to fish and other aquatic life when these are discharged into nearby water bodies (Ayyasamy *et al.,* 2008; Baruah *et al.*, 1993) simultaneously rendering water unfit for drinking and domestic purposes. These effluents have a high temperature which can be of concern because high temperatures reduce the level of dissolved oxygen (DO) levels in the water body. Effluents also bring out changes in the natural pH level of the water body to some extent. Dermatitis, skin disease and various other water borne diseases are major health problems resulting from the domestic use of eutrophied water (Nadia and Mahmood, 2006). The SME are also having a higher amount of suspended solids, dissolved solids, organic matters, press-mud, bagasse and cause air pollution also (Bevan, 1971; Hendrickson, 1971).

Non-stop use of SME harmfully affects the crops when used for irrigation. As a result, various elements including heavy metals get deposited in the soil and pollute it, and this polluted soil reduces both the quality of soil as well as the production of crops and also cause corrosion in water pipes (ETPI, 2001). As SME are commonly used for irrigation especially in fields which are in nearby areas of the sugar industry, it is essential to study the effect of irrigation using these effluents on crops. The effect has been studied by number of researchers, (Agarwal and Chaturvedi, 1995; Kumar and Rai, 2001; Saliha, 2003; Ramana, 2002).

Simultaneously using these waste waters has several benefits also in growth of some crops (Pandey *et al.*, 2007). After proper treatment, these wastewaters can be used for different purposes like agricultural irrigation. Srivastava *et al.*, 2017 have studied the effect of effluents by taking various concentrations of SME for irrigation of

V. unguiculata that soil microbial population can cause positive changes in soil health contaminated by heavy metals.

MATERIALS AND METHODS

Experimental Design (Collection of SME)

The effluents from Cooperative sugar industries located at Meham, Rohtak (28°59'49.2"N 76°14'30.1"E) of Haryana state (India) was collected in pre-cleaned, acid washed, 1L sample bottles from a settling tank installed in the sugar mill and stored in a refrigerator below 5°C until used.

Soil Sampling, and Analysis

The soil used for the experiment was collected from a depth of 0 to 15 cm. The soil is used after drying and sieving to remove debris. The soil was taken in different pots and was fertigated twice in a month with SME 25%, 50%, and 100% concentrations along with bore well water as the control. The different concentration of the effluents for bioassays, namely, control, 25%, 50%, and 100% was made by diluting the effluents with bore well water. The soil was analyzed before and after fertigation for various physicochemical parameters, namely, water holding capacity, soil texture, soil pH, EC, and OC following standard methods (Chaturvedi and Sankar, 2006).

Gram Staining

The most common technique is Gram staining. In differential staining technique, more than one stains are used which dye different types of microorganisms in different colors. The different colors obtained are due to inclusions within the cells or differences in the structure of cell wall. With the help of stains, different organisms can be identified. The most common differential stain is the Gram stain which divides bacteria into two groups: The bacteria which stain purple are Gram-positive and which stain pink are called Gram-negative.

RESULTS AND DISCUSSIONS

Soil Physico-chemical Parameters

The pH of the soil is found to be alkaline in nature (7.5–7.6), and it becomes more alkaline with an increase in the concentration of SME. In addition of more concentration of SME, there is an increase in EC of soil, which may be attributed to the addition of significant salts in the soil in the form of cations and anions. Various physico-chemical parameters of the soil are given in Table 1 and Figure 1.

Identification and Characterization of Microorganisms Present in the Soil

Identification and characterization of fungal isolates were carried out on the basis of colony growth (diameter), presence or absence of aerial mycelium, colony color, presence of furrows in the medium, pigment production, spore morphology, etc.

First identified fungus strain was *Aspergillus* sp. F1 shown in Figure 2, which was a filamentous fungus that is used in different food and enzyme industries. This fungus is characterized by a round vesicle with extending conidial chains, which appear as white and fluffy strands on the substrate that the fungus inhabits.

Taxonomic Classification

- Kingdom: Fungi
- Division: Ascomycota
- Class: Eurotiomycetes
- Order: Eurotiales
- Family: Trichocomaceae
- Genus: *Aspergillus*.

Second identified fungus was *Rhizopus* sp. F2 as shown in Figure 3, which is a cosmopolitan filamentous fungus generally isolated from soil, decaying fruits and vegetables, animal feces, and old bread. The *Rhizopus* species are also occasional causes of serious, even fatal, infections in humans. Certain species of this family are plant pathogens also.

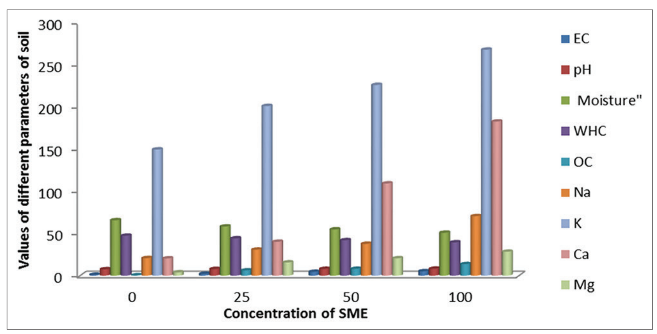

Figure 1: Variation of different parameters of soil with SME

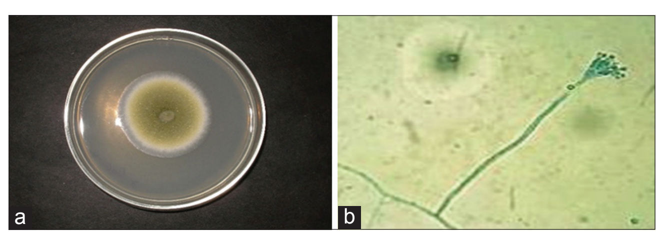

Figure 2: (a and b) First identified isolated fungus Aspergillus sp. F1 showing growth on agar plate and morphological features under a microscope (×100)

Table 1: Various physicochemical of soil with different concentration of SME

Parameter	Soil without SME	25% SME	50% SME	100% SME
EC (dS m^{-1})	1.10±0.02	2.13±0.02	4.06±0.03	5.36±0.04
pH	7.60±0.02	7.97±0.02	8.05±0.04	8.25±0.04
Soil moisture (%)	65.61±0.13	58.23±0.11	54.80±0.09	50.88±0.09
WHC (%)	47.37±0.07	44.14±0.04	41.96±0.03	39.45±0.04
OC (mg/kg)	0.49±0.02	6.27±0.04	8.15±0.04	13.72±0.04
Na^{+}	20.81±0.03	30.82±0.03	37.72±0.03	70.54±0.08
K^{+}	149.44±0.16	200.83±0.11	225.86±0.07	267.59±0.12
Ca^{+2}	20.46±0.06	40.14±0.05	109.19±0.06	182.53±0.05
Mg^{+2}	3.92±0.06	15.70±0.05	20.61±0.02	28.41±0.02

Taxonomic Classification

- Kingdom: Fungi
- Phylum: Zygomycota
- Order: Mucorales
- Family: Mucoraceae
- Genus: *Rhizopus.*

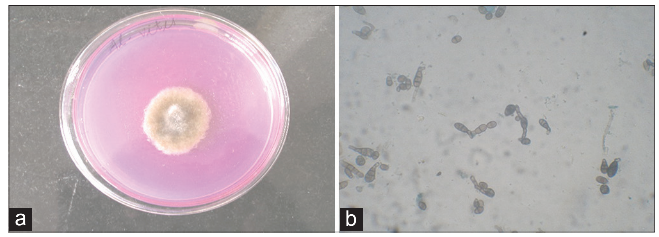

Figure 3: (a and b) Second identified isolated fungus Rhizopus sp. F2 showing growth on agar plates and morphological observations under a microscope (×100)

Alternaria sp. F3 as shown in Figure 4, is a fungus which has been recorded causing various diseases on plants. It is a pathogen which causes leaf spots, rots, and blights on many plant parts. This pathogen propagates itself through asexual spores called conidia.

Taxonomic Classification

- Kingdom: Fungi
- Division: Ascomycota
- Class: Dothideomycetes
- Order: Pleosporales
- Family: Pleosporaceae
- Genus: *Alternaria.*

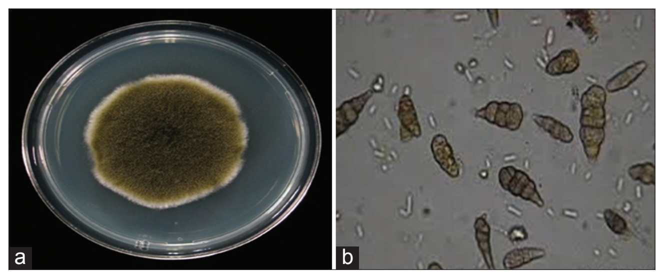

Figure 4: (a and b) Third identified isolated fungus Alternaria sp. F3 showing growth on agar plates and morphological observations under a microscope (×100)

On the basis of Gram staining technique, two bacterial isolates were found: One was Gram-positive and one was Gram-negative.

As shown in Figure 5, first isolated bacterial strain was identified as *Bacillus* sp. B1

Taxonomic Classification

- Domain: Bacteria
- Division: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Bacillaceae
- Genus: *Bacillus.*

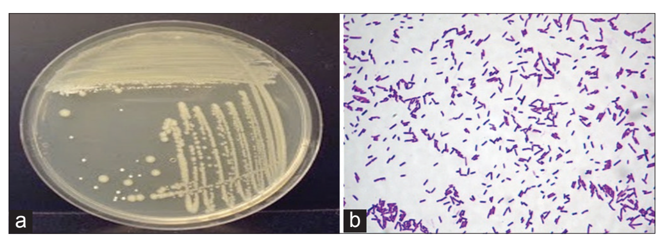

Figure 5: (a and b) First identified bacterial isolate Bacillus sp. B1 showing growth on agar plates and morphological features under a microscope (×100)

The second identified bacterial strain was *Staphylococcus* sp. B2 as shown in Figure 6. These are Gram-positive and spherical-shaped bacteria. *Staphylococcus* can cause a wide variety of diseases in humans and animals through toxin production or penetration.

Taxonomic Classification

- Domain: Bacteria
- Division: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae
- Genus: *Staphylococcus.*

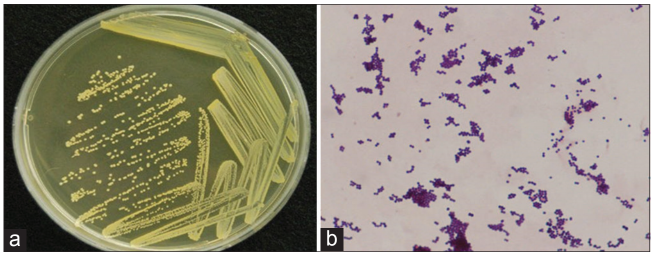

Figure 6: (a and b) Second identified bacterial strain Staphylococcus sp. B2 showing growth on agar plates and morphological features under a microscope (×100)

CONCLUSION

The present study confirms that with an increase in the concentration of SME, there is an increase in the concentration of salts in the soil and hence EC of such soil also increases. Similarly, increase in the concentration of heavy metals is also noticed, although it is within permissible limits. Various microorganisms are also noticed in the soil which may contribute to increase in quality of the soil for crops, but improved nutritional value of the soil for crops is maximum at a concentration of 50%. Hence, there is need of regular monitoring of the areas near the industries.

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